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TECHNICAL MANUSCRIPT 616

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TECHNICAL MANUSCRIPT 616

EFFECT OF WATER BALANCE AND AGE ON SPIRACULAR BEHAVIOR IN AEDES

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ABSTRACT

The behavior of the thoracic spiracles in female *Aedes triseriatus* was studied as a function of age. Duration of spiracular opening in air declined with sample age; in response to 0.5% CO_2, both amplitude and duration of opening declined; frequency of spiracular opening was not related to age. Aspects of feeding behavior were observed and related to spiracular behavior in *A. triseriatus* and *A. aegypti*. In the former species there was a diurnal weight loss; in both species inclusion of 0.2 M KCl in 0.3 M sucrose solution led to a reduction in the amount of fluid imbibed. Spiracular opening became conservative in *A. triseriatus* but not in *A. aegypti* when KCl was included in the diet. *A. aegypti* engorged to a greater extent than *A. triseriatus* and showed less conservative spiracular behavior. The course of starvation was more rapid in *A. triseriatus* than in *A. aegypti*; the rate of water loss in the former was four times that in the latter. Both species showed increasingly conservative spiracular behavior as starvation proceeded.

I. INTRODUCTION*

Because of the great ratio of surface area to volume in insects, their water-conserving mechanisms are of considerable importance to survival. Occlusible spiracles are one feature whereby water loss through respiratory transpiration is reduced.[1] Low relative humidities seem to accelerate the mortality rate of caged mosquito populations.[2,3] Similarly, field populations of *Anopheles gambiae* and *Anopheles funestus* suffer a 10% higher daily mortality rate in the dry season than they do in the wet season.** These observations suggest that mosquitoes, quite in contrast to *Glossina*,[4,5] have a poorly developed ability to regulate water loss in a xeric environment.

The work presented here explores some relationships between desiccation and spiracular behavior in *Aedes* (*Finlaya*) *triseriatus* (Say) and *Aedes* (*Stegomyia*) *aegypti* (L.). The idea that the potassium ion might play an important part in the regulation of spiracular behavior as advocated by Hoyle[6] and Miller[7] was also investigated. Observations were made on the course of desiccation (starvation) and on water balance. Because a fluctuation in water balance with age was noted, spiracular behavior was investigated as a function of this variable.

II. MATERIALS AND METHODS

Techniques and apparatus used for observing and recording spiracular behavior were described in detail elsewhere; rearing and handling procedures of the biological material were also previously outlined.[8]

To study spiracular behavior as a function of age, 500 female adult *A. triseriatus* were maintained in gallon ice-cream cartons at about 26 C and 80% relative humidity and given constant access to 30 cc of 10% sucrose solution in cotton pads provided fresh each morning. All solutions were made with deionized water. Ten to 15 mosquitoes were individually sampled between 1330 and 1600 hours on each of days 1 through 5 and 8 through 12 of adult life. Each insect was observed in air (1 minute) and in air plus 0.5% CO_2 (2 minutes) at 21.1 to 22.5 C and 90±5% relative humidity. Mosquitoes were allowed a 2-minute accommodation period to the experimental conditions before observation. The 2-week series of observations was performed three times.

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Krafsur, E.S. 1969. Unpublished observation.

III. RESULTS

A. SPIRACULAR BEHAVIOR AS A FUNCTION OF AGE

Mosquitoes were 1 day old when first observed and had been given access to sucrose solution. The daily observation averages are graphically shown in Figures 1 and 2. These clearly indicate that spiracular behavior became more conservative with increasing age when mosquitoes were observed in air and in air plus 0.5% CO_2.

The duration of spiracular opening in air (Fig. 1) declined at a steady rate over the 2-week aging period; the frequency of opening showed no significant or uniform change. The slope of duration of opening was 1.1 sec/min per day. Presumably, high sampling variation resulted in insignificant differences in duration within weeks; however, the difference between weeks was highly significant (Table 1).

When expressed as a function of age, responses to CO_2 (Fig. 2) were basically similar to those for air alone. However, a linear rate of decline in amplitude and duration was not suggested; rather, decline in these became attenuated with advancing age. Again there was no significant difference within weeks, but between-week differences in mean measurements of amplitude and duration of spiracular opening were highly significant (Table 2). The average frequency of spiracular opening in CO_2 showed even greater day-to-day variation than that in air alone, and no significant differences were found on either a daily or a weekly basis. Plots of the mean frequency of opening were, despite greater variation in CO_2, approximately congruent with those in air.

The relationships of duration to amplitude of opening were fairly constant with one another both in air and in CO_2 throughout the aging period.

B. RELATIONSHIPS BETWEEN SPIRACULAR BEHAVIOR AND ADVANCING DESICCATION

Preliminary information was required about the pattern of mosquito water balance. Differences in fresh wet weight were chosen as an index of water balance. The following experiment was designed to measure the wet weights of *A. triseriatus* and *A. aegypti* from emergence through the 1st week of adult life. One additional group of *A. triseriatus* was observed over a 2-week period. With this information, spiracular behavior of desiccated (starved), KCl-fed, and normally fed (control) groups of insects could be assessed more adequately. Pint cartons of 50 *A. triseriatus* or *A. aegypti* each were randomly distributed throughout an incubator. Each morning, fresh pads of 30 cc of either 10% sucrose (0.3 M) or 0.2 M KCl in 10% sucrose were placed on the appropriate cartons. At 0800 and 1400 hours each day one container of each treatment was randomly selected and frozen to kill the mosquitoes for weight determinations. This set of observations was repeated three times.

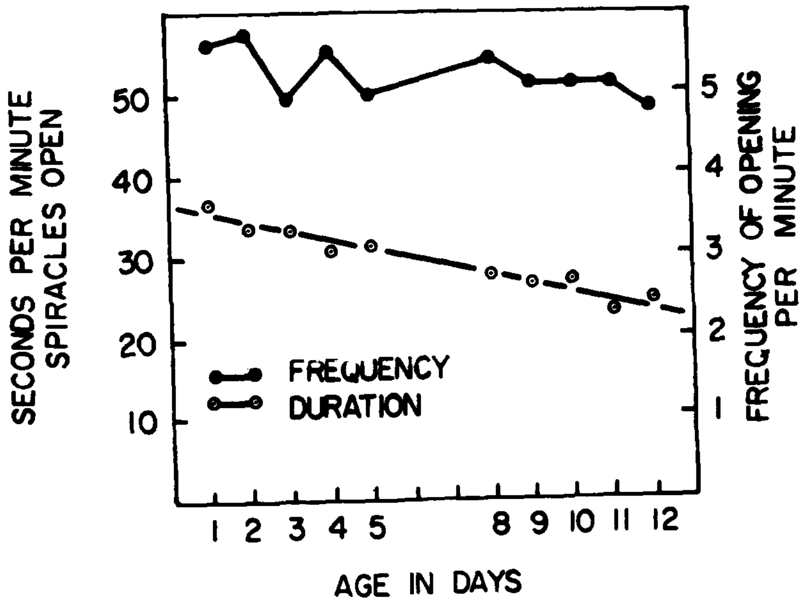

FIGURE 1. Frequency and Duration of Spiracular Opening in *Aedes triseriatus* as a Function of Age. Mosquitoes were observed in air.

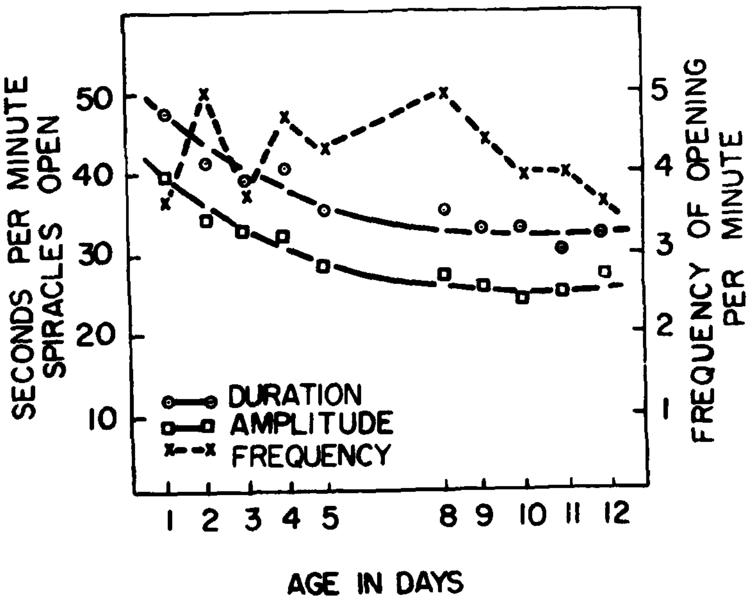

FIGURE 2. Frequency, Duration, and Amplitude of Spiracular Opening in *Aedes triseriatus* as a Function of Age. Mosquitoes were observed in an atmosphere of air plus 0.5% CO_2.

TABLE 1. SPIRACULAR BEHAVIOR IN *AEDES TRISERIATUS* OBSERVED IN AIR AND IN AIR PLUS 0.5% CO_2 AS A FUNCTION OF AGE IN WEEKS[a]

	Air		Air Plus 0.5% CO_2			
	Freq[b]	Dur[c]	Freq	Ampl[d]	Dur	N[e]
Week 1	5.39	33.28	4.27	34.08	41.15	154
Week 2	5.12	25.93[f]	4.22	26.03[f]	33.05[f]	175

a. This experiment was repeated three times.
b. Freq = frequency, number of spiracular openings from closed position per minute.
c. Dur = duration of spiracular opening, seconds per minute open at all magnitudes.
d. Ampl = amplitude of spiracular opening, seconds per minute completely (or nearly so) open.
e. Number of observations.
f. $P<0.005$.

TABLE 2. SPIRACULAR BEHAVIOR IN THREE SUCCESSIVE GROUPS OF *AEDES TRISERIATUS* OBSERVED IN AIR AND IN AIR PLUS 0.5% CO_2[a]

	Air		Air Plus 0.5% CO_2				Mean Wet Eclosion Weight, mg
	Freq	Dur[b]	Freq	Ampl[c]	Dur	N	
Group 1	5.35	29.08	4.72	27.11	35.45	107	2.63
Group 2	5.31	30.17	4.26	30.10	37.27	119	2.69
Group 3	5.06	28.76	3.73[d]	32.24	37.78	103	2.18

a. Stated values are means from samples collected daily over a 2-week period.
b. Dur = duration of spiracular opening.
c. Ampl = amplitude of spiracular opening.
d. $P<0.005$.

Initial eclosion weights of A. triseriatus were greater than those of A. aegypti; in these particular experiments, A. triseriatus weighed an average 5% more than A. aegypti; after allowing access to sucrose solution for each of 5 days, the average value changed to 83.5% of the fresh wet weight of A. aegypti. Thus, at least during the photophase, A. aegypti engorge to a much greater extent than A. triseriatus. Six hours following initial access to sucrose, A. triseriatus gained 18% of their unfed weight; after 24 hours weight gains were 26%. Thereafter they lost weight between the morning and afternoon sampling periods with considerable periodicity, the average value being 7.4% (Table 3).

Six hours after the first opportunity to engorge, A. aegypti gained 49% of their original unfed weight and this increased to 59% after 24 hours. Periodic fluctuation in weight was not so marked in sucrose-fed A. aegypti, amounting to an average weight loss of only 1.7% from morning until afternoon. However, diurnal loss in weight was greater in the KCl-fed group. These data suggest that A. aegypti probably feed throughout the day, but A. triseriatus does not engorge enough to maintain its own weight during the photophase.

The addition of 0.2 M KCl to the sucrose diet offered to populations of mosquitoes depressed the total amount of fluid ingested (Table 3). Thus, weight of KCl-fed A. aegypti averaged 91.6% of weight of groups fed only sucrose; KCl-fed A. triseriatus weights were only 86.2% of the control weight, a considerably greater effect.

The course of starvation in A. triseriatus was uniform among different age groups. Eight hours after withdrawal of nutrient, mosquitoes weighed 90 to 95% of fed controls; 24 to 30 hours after sucrose was denied, weights were 70 to 75% of fed populations. Average weight loss was 0.04 to 0.05 mg/hour. Mortality exceeded 95% after 48 hours of starvation. Starvation in A. aegypti took remarkably longer, a fact related to the much greater amount of sucrose solution this species normally imbibes and an apparently lower rate of weight loss. Thus, this species lost 0.01 to 0.015 mg/hour when denied nutrient. Expressed in terms of percentage loss, 4 days of starvation resulted in a 35 to 45% reduction in weight; after 6 such days the values became 58%. At this point, the mortality was only 40% of the original population.

Two series of observations were made on the behavior of the spiracular valves throughout the course of starvation in A. triseriatus (Table 4). In the first series, nutrient was withheld from 3-day-old mosquitoes (day 1 of starvation). During the first 8 hours of starvation, the duration of spiracular opening in air was 73% of that in the fed control group. In CO_2, amplitude of spiracular opening was 80% and duration 86% of those values in the unstarved controls. Between 24 and 30 hours of starvation, duration of opening in air was only 64% of those allowed constant access to sucrose solution; in response to CO_2, the amplitude of spiracular opening in starved mosquitoes was 13% of that in fed insects and the duration of such opening was 69% of the response of presumably well-hydrated individuals.

TABLE 3. FRESH WET WEIGHTS OF *AEDES* ALLOWED TO FEED ON 0.3 M SUCROSE OR 0.3 M SUCROSE PLUS 0.2 M KCl[a]

	Day 1		Day 2		Day 3		Day 4		Day 5	
	a.m. (unfed)	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
A. aegypti										
Sucrose + KCl	2.31	3.27	3.45	3.08	3.33	3.07	3.37	3.17	3.46	3.11
Weight Diff.[b]	+41.5%		-10.7%		-7.5%		-6.0%		-10.0%	
Sucrose	2.30	3.43	3.65	3.46	3.50	3.39	3.64	3.54	3.65	3.61
Weight Diff.[b]	+49%		-5.2%		-3.1%		-2.8%		-1.1%	
A. triseriatus										
Sucrose + KCl	2.43	2.46	2.68	2.56	2.73	2.45	2.66	2.41	2.55	2.39
Weight Diff.[b]	+1.2%		-4.5%		-10.2%		-9.5%		-6.5%	
Sucrose	2.43	2.68	3.06	2.78	3.15	2.80	3.04	2.86	3.06	2.95
Weight Diff.[b]	+18.5%		-9.2%		-11.1%		-6.9%		-3.6%	

a. Each value is the average weight in milligrams of 150 mosquitoes.
b. Difference in a.m. and p.m. weights expressed as a percentage of the former.

TABLE 4. SPIRACULAR BEHAVIOR IN STARVED AND FED *AEDES TRISERIATUS* AT 26.6 C AND 80% RH

Day of Starvation	Status	No. Observed	Air		Air + 0.5% CO_2				Avg Weight, mg
			Freq	Dur[a]	Freq	Ampl[b]	Dur	Activity,[c] %	
3- and 4-day-old mosquitoes									
Day 1	Fed	10	5.0	27.3	4.2	31.0	36.8	0	2.97
	Starved	10	4.3	20.0	4.9	24.8	31.5	33	2.77
Day 2	Fed	10	6.1	32.6	3.3	35.2	42.1	12	2.97
	Starved	10	6.5	20.9	6.2	4.7	28.9	12	2.15
9- and 10-day-old mosquitoes									
Day 1	Fed	11	5.5	30.9	4.0	33.5	30.4	18	3.31
	Starved	11	4.4	25.1	4.0	24.8	35.9	36	2.90
Day 2	Fed	11	4.5	28.4	3.7	26.7	35.9	18	3.25
	Starved	11	5.2	25.2	5.0	12.8	30.6	25	2.68

a. Dur = duration of spiracular opening.
b. Ampl = amplitude of spiracular opening.
c. Percentage of sample showing spontaneous locomotor activity.

The second series consisted of older A. triseriatus. Starvation was begun early on the 9th day of adult life and continued throughout the 10th (Table 4). The results were similar to those obtained in the first experiment, i.e., spiracular opening became more conservative as water reserves were depleted. In both series the frequency of spiracular opening was greater in the starved groups, a fact that may suggest an increased frequency of motor impulses from the central nervous system to the spiracular closer muscle.[9-11] Starved mosquitoes showed a slightly elevated degree of "spontaneous locomotor activity," a phenomenon that would ordinarily increase spiracular opening.[8]

Attention was next turned toward A. aegypti. Nutrient was withheld from 8-day-old mosquitoes and observations were continued for the following 5 days. Weight loss was measured throughout the latter period. Weights of starved mosquitoes were about 86%, 83%, 76%, and 68% of sucrose-fed controls on days 2 through 5. Thus, A. aegypti never reduced to their emergence weight in this particular experiment, since this mosquito normally maintains a third or more of its weight in sucrose solution. Spiracular behavior during the first 2 days of starvation was similar to that in mosquitoes given constant access to sucrose solution. On days 3 and 4, however, starved insects showed more conservative spiracular opening; amplitude was 75% of that of the fed controls. On day 5 of starvation there was again little difference in spiracular behavior between starved and fed mosquitoes. Spontaneous locomotor activity was consistently greater in the starved group. Unfortunately, it was impossible to continue observations beyond the 5th day of starvation, although mortality in this treatment was only about 2%.

A second experiment with A. aegypti was designed to evaluate spiracular behavior in individuals starved to a point where mortality reached approximately half the population (Table 5). Starvation was begun early on the 3rd day of adulthood and continued for 6 additional days, a point at which 68% of the population survived versus 98% of the fed controls. Mosquitoes weighed an average 69%, 56%, 53%, and 40% of fed controls over days 4 through 7 of starvation. It is remarkable that, over the final 4 days of starvation, the rate of weight loss varied only 0.014 to 0.012 mg/hour. Spiracular behavior was observed on the 4th through 7th days of starvation. Advancing desiccation resulted in an increasing attentuation of responses to CO_2; in air there was no significant difference in the duration of spiracular opening although the frequency of such opening was usually greater, a phenomenon observed in responses to CO_2. Noteworthy is the greater spontaneous activity in the starved group of mosquitoes, for this is associated with increased spiracular opening and tends to counteract the conservative spiracular behavior effect of desiccation.

TABLE 5. SPIRACULAR BEHAVIOR IN CONTINUOUSLY STARVED AND CONTINUOUSLY FED *AEDES AEGYPTI*[a]

Day of Starvation	Status	No. of Observations	Air		Air + 0.5% CO_2				Weight,[c] mg
			Freq	Dur	Freq	Ampl	Dur	Activity,[b] %	
Day 4	Fed	6	6.0	26.0	2.2	44.2	47.6	67	2.98
	Starved	6	6.2	28.0	4.0	30.0	36.4	100	2.06
Day 5	Fed	9	5.8	28.7	2.9	51.2	54.8	78	2.98
	Starved	9	4.9	27.1	2.8	33.6	48.6	100	1.66
Day 6	Fed	10	4.7	24.7	3.1	44.0	45.2	40	2.98
	Starved	10	6.7	32.7	5.2	23.9	36.9	100	1.57
Day 7	Fed	10	5.3	30.1	2.5	38.0	46.9	70	2.98
	Starved	10	5.5	28.5	3.9	16.4	35.3	60	1.20

a. Mosquitoes were 3 days old when starvation was begun and maintained at 26.6 C and 80% RH.
b. Percentage of sample showing spontaneous locomotor activity.
c. Eclosion (unfed) average weight was 1.58 mg.

C. THE EFFECTS OF KCl-SUCROSE DIET ON SPIRACULAR BEHAVIOR

Since inclusion of 0.2 M KCl in the normal sucrose diet reduced the average amount of water reserves in A. triseriatus by 14% and in A. aegypti by only 8%, it might be expected that spiracular behavior in air would be more conservative than spiracular responses to CO_2. On the premise that the adult gut is freely permeable to the potassium ion, another tenable hypothesis is that internally raised potassium levels might decrease the resting potential and slightly depolarize the spiracular closer muscle. Tighter control of the spiracle would result, as it does in Schistocerca.[6] Miller[7] showed that increased internal potassium may act directly on the spiracle motor nerves, depolarizing them to a small extent, thus increasing their rate of firing. This also increases spiracular control and is manifested by raised thresholds of response to CO_2 and hypoxia in dragonflies.[7]

The effects of potassium on spiracular behavior in air and in response to CO_2 were tested by comparing A. triseriatus fed KCl in sucrose with sucrose-fed controls. The mosquitoes were 1 to 4 days old. The results are given in Table 6. Although spiracular control was greater in the KCl-fed group, frequency of opening was lower. Thus, the duration of each spiracular opening was greater but each closed period was longer in the KCl-fed mosquitoes. Variation in duration and amplitude of spiracle opening was less in the sucrose-fed controls. These findings are not incompatible with an increased rate of motor nerve impulse frequency to the spiracular closer muscle or increased tension and contracture in the muscle itself as described by Hoyle[6] and Miller.[7] On the other hand, in Aedes more conservative spiracular opening is usually associated with increased frequency of opening (and hence, closing).

A similar experiment used 2- through 5-day-old adult female A. aegypti (Table 7). KCl-sucrose-fed specimens showed only a slight and insignificant reduction in response to CO_2, and variation was again greater in the experimental group. Thus, neither the potassium ion nor the slight reduction in weight influenced control of the spiracles.

TABLE 6. SPIRACULAR OPENING IN *AEDES TRISERIATUS* FED EITHER 0.2 M KCl IN 10% SUCROSE OR SUCROSE ALONE

	Air		Air + 0.5% CO_2			No. of Observations
	Freq ± S.E.	Dur ± S.E.	Freq ± S.E.	Ampl ± S.E.	Dur ± S.E.	
0.2 M KCl	2.6 ± 0.18	40.7 ± 1.5	2.1 ± 0.17	21.7 ± 1.8	45.2 ± 1.3	130
Sucrose	3.5 ± 0.22	42.6 ± 1.3	2.5 ± 0.20	29.6 ± 1.3	49.8 ± 1.0	130

TABLE 7. THE EFFECTS OF 0.2 M KCl IN 10% SUCROSE ON SPIRACULAR OPENING IN *AEDES AEGYPTI*

	Air		Air + 0.5% CO_2			No. of Observations
	Freq ± S.E.	Dur ± S.E.	Freq ± S.E.	Ampl ± S.E.	Dur ± S.E.	
0.2 M KCl	3.9 ± 0.21	46.2 ± 1.1	1.7 ± 0.11	54.8 ± 0.9	57.4 ± 0.5	100
Sucrose	4.0 ± 0.21	44.2 ± 1.1	1.7 ± 0.11	56.1 ± 0.5	58.2 ± 0.3	100

IV. DISCUSSION

Daily observations on spiracular behavior in A. triseriatus suggested that variation was great enough to mask an otherwise pronounced influence of age. The latter was statistically quite apparent on a weekly basis. Although Bat-Miriam and Galun[12] reported great variation in O_2 uptake in A. aegypti that were sampled daily, there was an increase in the respiratory rate over the first 3 days of adult life. Miller[7] noted marked variation in the degree of spiracular control in wild-caught dragonflies. The present work showed that both the duration and magnitude of spiracular opening declined with sample age, as did response to CO_2. An explanation for the decline is obscure. Once allowed access to nutrient, there was no steady gain or loss of weight over the 2-week period, although there was a daily fluctuation. Thus it is unlikely that attenuation in spiracular opening was a result of variation in water balance. The frequency of spiracular opening was the most variable of all features observed. Galun and Fraenkel[13] suggested it varied inversely with barometric pressure, which perhaps may account for the present findings.

Potassium chloride depressed the amount of fluid imbibed by Aedes, a feature that may be directly related to the osmotic strength of the nutrient solution. The rate of crop emptying in cockroaches[14] and adult Phormia regina[15] was shown to be inversely proportional to the osmotic concentration of the ingested solution.

Greater spontaneous locomotor activity and looser control of the spiracles was observed in A. aegypti than in A. triseriatus.[8] Spiracular behavior was affected to a lesser extent by low relative humidity in A. aegypti than in A. triseriatus.[16] An explanation is that the former species can afford higher rates of transpiration because of greater reserves of fluid. If true, then why were rates of water loss apparently four times higher in starving A. triseriatus? Bursell[4] noted that in resting Glossina adults, only 25% of the water loss occurred via spiracular transpiration. Although it is perhaps unlikely, the cuticle of A. triseriatus may be more permeable to the outward diffusion of water than that of A. aegypti at the particular temperatures of this experiment. Conceivably, A. triseriatus may take in fluids at the same rate as A. aegypti, but excrete them much more rapidly. This would imply a faster rate of crop emptying and elimination from the gut—if in fact spiracular transpiration is not responsible for the high rate of water loss in this species. However, A. aegypti at rest, in flight, in hypoxic and in CO_2-containing atmospheres, and when locomotor activity was eliminated always showed more spiracular opening than similarly treated A. triseriatus.

The rate of water loss in Glossina declines as water reserves become depleted.[4] In A. aegypti this value only declined from 0.014 mg/hour on day 4 of starvation to 0.012 mg/hour on day 7. Nevertheless, despite greater locomotor activity under the conditions of starvation presently imposed, Aedes demonstrated increasingly attenuated spiracular opening with progressive water loss.

Miller[7] demonstrated a correlation between the amount of spiracle opening in dragonflies and their state of hydration. Further efforts showed that motor impulse frequency to the spiracular closer muscle was higher in desiccated specimens and could be duplicated by perfusion of the thorax and abdomen with salines of greater than normal ionic concentration. Miller suggested that this provided the mechanism whereby water balance influenced the degree of spiracular opening, i.e., hemolymph ion concentration varies inversely with water reserves. In the present work, one hypothesis can best explain the effect of 0.2 M KCl in sucrose solution on spiracular behavior in _Aedes_: the reduction in spiracular opening seen in _A. triseriatus_ was a consequence of reduced fluid intake with a resulting mild desiccation. Potassium chloride did not affect spiracular behavior in _A. aegypti_ because its fluid intake, although depressed, remained at such a level that no desiccation occurred. To invoke a specific pharmacologic effect of potassium ion would require a significant change in spiracular opening in this species.

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13. ABSTRACT

The behavior of the thoracic spiracles in female *Aedes triseriatus* was studied as a function of age. Duration of spiracular opening in air declined with sample age; in response to 0.5% CO_2, both amplitude and duration of opening declined; frequency of spiracular opening was not related to age. Aspects of feeding behavior were observed and related to spiracular behavior in *A. triseriatus* and *A. aegypti*. In the former species there was a diurnal weight loss; in both species inclusion of 0.2 M KCl in 0.3 M sucrose solution led to a reduction in the amount of fluid imbibed. Spiracular opening became conservative in *A. triseriatus* but not in *A. aegypti* when KCl was included in the diet. *A. aegypti* engorged to a greater extent than *A. triseriatus* and showed less conservative spiracular behavior. The course of starvation was more rapid in *A. triseriatus* than in *A. aegypti*; the rate of water loss in the former was four times that in the latter. Both species showed increasingly conservative spiracular behavior as starvation proceeded.

14. Key Words

Water balance
Age
Spiracles
Spiracular behavior
Aedes aegypti
Aedes triseriatus
Starvation
Desiccation
Potassium
Carbon dioxide
Respiratory system

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